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BIOTRANSFORMATION OF TWO *ENT*-15β-HYDROXY-KAUR-16-ENE DERIVATIVES BY *GIBBERELLA FUJIKUROI*

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Key Word Index—*Gibberella fujikuroi*; diterpenes; *ent*-15β-hydroxy-kaur-16-ene; candidiol; microbiological transformations.

Abstract—Incubation of the fungus *Gibberella fujikuroi* with *ent*-15β-hydroxy-kaur-16-ene gave *ent*-11α,15β-dihydroxy-kaur-16-ene, *ent*-7β,11α,15β-trihydroxy-kaur-16-ene, *ent*-11α,13,15β-trihydroxy-kaur-16-ene, *ent*-11α,15β,19-trihydroxy-kaur-16-ene, and *ent*-11α,14α,15β-trihydroxy-kaur-16-ene, and a mixture of products, which was resolved by acetylation to give *ent*-11α,14α,15β-triacetoxy-kaur-16-ene and *ent*-7β,15β,17-triacetoxy-11α,16α-epoxy-kaur-16-ene. The addition of candidiol (*ent*-15β,18-dihydroxy-kaur-16-ene) gave *ent*-11α,15β,18-trihydroxy-kaur-16-ene, and a mixture of substances, which was resolved by acetylation to give *ent*-11β,15β,18-triacetoxy-kaur-16-ene, *ent*-7β,11α,15β,18-tetraacetoxy-kaur-16-ene and *ent*-15β,17,18-triacetoxy-11α,16α-epoxykaurane. These results confirm that the presence in *ent*-kaur-16-ene derivatives of a 15α-hydroxyl group inhibits oxidation at C-19 to the acid level. The biotransformation of these compounds may be useful for the synthesis of natural 11β-hydroxy-*ent*-kaurene analogues.

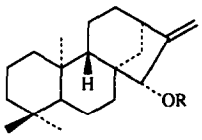
INTRODUCTION

In previous studies, we have shown that several 15α-hydroxy-*ent*-kaurene derivatives are hydroxylated at C-11β by the fungus *Gibberella fujikuroi*, the presence of the 15α-hydroxy group inhibiting oxidation at C-19 [1–3]. This oxidation is characteristic of the biosynthesis of gibberellins and kaurenolides [4]. We have now incubated *G. fujikuroi* with 15α-hydroxy-*ent*-kaur-16-ene (**1**), the least polar of the *ent*-kaurene derivatives hydroxylated at C-15. We have also completed our previous work on the microbiological transformation of candidiol (**3**), another 15-hydroxy derivative, by this fungus, obtaining further information about the substrate specificity of the enzymes involved in the biosynthesis of gibberellins.

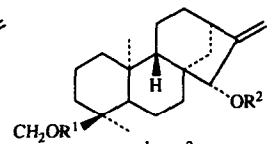
RESULTS AND DISCUSSION

The diterpene **1** was prepared from candidiol (**3**), which had been isolated from species of the genus *Sideritis* [5, 6] endemic to the Canary Islands. Thus, the diacetate of candidiol (**4**) was partially hydrolysed to give the monoacetates **5** and **6**. Compound **5** was treated with triphenylphosphine in carbon tetrachloride to give the chloride **7**. Hydrolysis of **7** afforded compound **8**, and this was reduced with tri-*n*-butyl tin hydride to afford the required compound **1**. The substrate **1** was also obtained starting from **9**. Treatment of this compound with triphenylphosphine-carbon tetrachloride gave the chloro-derivative **10**, which was reduced as above to afford *ent*-kaur-16-ene (**11**). Allylic oxidation of **11** with SeO_2 gave **1**. The fermentation was carried out in the presence of AMO 1618, a compound that inhibits the formation of *ent*-kaur-16-ene (**11**) without perturbing post-kaurene metabolism [7, 8].

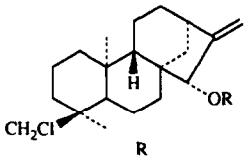
One of the compounds (**12**) obtained in the fermentation possessed one more oxygen than the substrate **1**. Its ^{1}H NMR spectrum was similar to that of **1**, except that a new hydrogen geminal to a hydroxyl group appeared in the spectrum at δ3.89 (J = 5 Hz). The chemical shift and



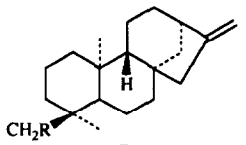
R
1 H
2 Ac



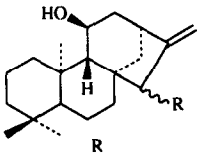
R^1	R^2
3 H	H
4 Ac	Ac
5 H	Ac
6 Ac	H



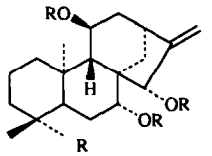
R
7 Ac
8 H



R
9 OH
10 Cl
11 H



R
12 α OH
13 β OH



R
14 H
15 Ac

the coupling constant of this proton were similar to those assigned to the hydrogen at C-11 in **20**, which was formed in the incubation of **21** with *G. fujikuroi* [2]. Thus the structure *ent*-11α,15β-dihydroxy-kaur-16-ene (**12**) was assigned to the least polar compound. Its 15-epimer, **13**, has been isolated from the liverwort *Solenostoma triste* [9].

Another compound isolated in this incubation was identified as *ent*-7β,11α,15β-trihydroxy-kaur-16-ene (**14**) as follows: its ^{1}H NMR spectrum possessed a complex signal at δ3.92 due to the geminal hydrogens of two secondary alcoholic groups. Acetylation of this product under the usual conditions, formed a triacetate (**15**). Its ^{1}H NMR spectrum in chloroform-*d* showed one of the geminal protons to an acetylated hydroxyl group as a quartet centred at δ4.88 with coupling constants of 7 and 11 Hz, which is typical of an equatorial substituent at C-1, C-3 or C-7. Positions 1 and 3 were excluded from a consideration of the ^{13}C NMR data (Table 1). In the ^{1}H NMR spectrum, the geminal hydrogen to a hydroxyl group at C-11 appeared overlapped with one of the protons at C-17, and when this spectrum was run in benzene-d_6 the hydrogen at C-11 overlapped with the other hydrogen at C-17. Finally, in a mixture of these two solvents the resonance of this hydrogen could be observed as a doublet with a coupling constant of 6 Hz. This form of resonance and the chemical shifts of this hydrogen in **14** and in its acetate **15**, at δ3.92 and 5.12, respectively, are typical of the geminal hydrogen at C-11β to these two oxygen functions [1–3, 9].

The third compound, **16**, obtained in this incubation had the molecular formula $C_{20}H_{32}O_3$, and possessed two oxygen atoms more than the substrate **1**. The ^{1}H and ^{13}C NMR spectra of its triacetate **17** indicated that these two oxygens were introduced into the molecule as two new hydroxyl groups, one tertiary and the other secondary. The geminal hydrogen to the secondary hydroxyl group appears in the ^{1}H NMR spectrum of **16** as a doublet (J = 5.5 Hz) at δ4.10 and was assigned to C-11β for the same reasons as in the case of **14**. The tertiary alcohol function was assigned to C-13 taking into consideration its facile acetylation, in comparison with other tertiary alcohol groups possible in this skeleton, and the lack of the H-13 signal and the resonance of the two hydrogens at C-17 in its ^{1}H NMR spectrum [3]. Moreover, the ^{13}C NMR spectrum of **17** (Table 1) is in accordance with this structure.

Compound **18** was also obtained in this incubation. Its HR mass spectrum was in accord with the formula $C_{20}H_{32}O_3$. The two new oxygens of the molecule belong to two secondary alcohols. The geminal protons appearing in the ^{1}H NMR spectrum at δ3.77 (*d*, J = 3.5 Hz) and 4.04 (*br s*) were assigned to C-11 and C-14, respectively. The chemical shifts and the form of resonance are typical of hydrogens geminal to a hydroxyl group at C-11β (see above) and C-14β [10]. The ^{13}C NMR spectrum of the triacetate **19** (Table 1) is also in accordance with the structure assigned to this compound.

Another substance obtained in this experiment was the triol **20**. Its ^{1}H NMR spectrum showed the presence of two new hydroxyl groups, one primary and the other secondary, which were assigned to C-19 and C-11β, respectively. Product **20** was identical with a compound formed in the microbiological transformation of *ent*-15β,19-dihydroxy-kaur-16-ene (**21**) by *G. fujikuroi* [2].

The most polar compound isolated in this fermentation was separated as the triacetate **23** by acetylation of the fractions that contained it, and subsequent chromatography. Its ^{1}H NMR spectrum showed that the substrate had been transformed into a substance with new oxygen functions at C-7α, C-11β, C-16 and C-17. The three acetylated hydroxyl groups were assigned to C-15β,

Table 1. ^{13}C NMR spectral data of compounds **1**, **2**, **5**, **6**, **15**, **17**, **19**, **23**, **35** and **37** (50.32 MHz, except for **1** and **5** which were measured at 20.15 MHz)

C	1	2	5	6	15	17	19	23	35	37
1	40.5	40.4	39.7	39.7	39.8	40.0	39.9	41.1	40.6	39.8
2	18.7	18.6	17.9	17.7	18.4	18.5	18.4	18.4	17.7	17.5
3	42.1	42.0	35.0	35.6	41.6	41.8	41.8	41.5	36.7[a]	35.7
4	33.2	33.3	37.3	36.3	33.3	33.5	33.4	33.9	36.8	36.5
5	56.2	55.8	48.8	49.7	51.3	55.8	55.4	51.7	51.3	45.0
6	19.4	19.3	18.8	19.2	26.4	19.1	19.1	26.4	17.8	26.2
7	35.2	37.4	37.1	34.7	73.3	34.2	31.3	73.6	37.9	72.7
8	47.7	47.4	47.1	48.8	50.1	45.8	45.8	51.8	46.3	50.1
9	54.4	54.0	53.6	54.1	60.8	59.3	60.8	58.9	53.2	60.7
10	39.6	39.6	39.2	39.2	38.4	38.6	38.9	36.9	37.9	39.8
11	18.0	18.2	17.6	17.9	68.1	69.7	70.3	77.2	71.3	68.1
12	32.8	32.9	32.7	32.6	39.6	42.7	34.0	40.5	36.8[a]	39.2
13	42.4	42 7	42.4	42.2	39.9	84.6	45.4	40.7	40.9	39.9
14	36.4	34.7	34.1	36.2	30.7	39.7	76.2	33.1	33.0	30.7
15	83.0	83.4	83.1	82.7	81.4	80.3	83.1	84.2	82.8	81.2
16	160.5	155.7	155.4	160.1	154.9	153.1	149.8	88.7	156.2	156.8
17	108.0	109.9	109.7	108.2	109.2	110.9	114.0	63.4	111.8	109.3
18	33.6	33.8	71.9	72.7	33.3	33.7	33.7	33.4	74.2	72.0
19	21.7	21.7	17.3	17.4	21.8	22.2	21.6	21.5	18.3	17.7
20	17.7	17.7	17.7	18.0	17.3	17.5	16.0	18.7	19.1	17.7

[a]These values may be interchanged.

present in the substrate, C-7α and C-17 as follows. The geminal hydrogen of the acetate at C-7 appeared in this spectrum at a similar position and with the same coupling constants as in that of the triacetate **15**, also described in this work. The presence of the *gem*-dimethyl group at C-4 and the disappearance of the exocyclic double bond of the substrate were evident and, therefore, the acetoxymethylene group was located at C-17. The type of resonance, a pair of doublets at δ3.97 and 4.49, indicated that the C-16 position was also substituted by an oxygen function. Finally, the presence of another oxygen function at C-11β was detected by the resonance of its geminal hydrogen (δ4.43, *t*) which was similar to that described by us for **27**, also obtained by acetylation of one of the products (**26**) isolated as a biotransformation product of *ent*-7β,15β-dihydroxykaur-16-ene [3]. Taking into consideration this data, the structure of this triacetate can be formulated as **23** or **25**, and the corresponding alcohols as **22** or **24**. The formation of a triacetate and the absence of free hydroxyl groups in the IR spectrum permitted the structure **22** to be assigned to this compound, with an ether bridge between C-11β and C-16.

Compound **22** must be an artefact produced in the treatment of the ethyl acetate extract with aqueous acid (see Experimental) by attack of the C-16 hydroxyl group of the true biotransformed substance (**30**). The oxirane ring in this product must have α-stereochemistry because the epoxidation of *ent*-kaurene derivatives occurs via the α-face. On the other hand, the stereochemistry at C-16 of the ether bridge in **22** was assigned as β because hydroxy group attack to this centre is by the β-face, also favoured by the opening of the α-oxirane ring. Products with an ether bridge between C-11 and C-16 have been obtained by acid treatment of 11β-hydroxy-*ent*-kaur-16-ene derivatives [11, 12].

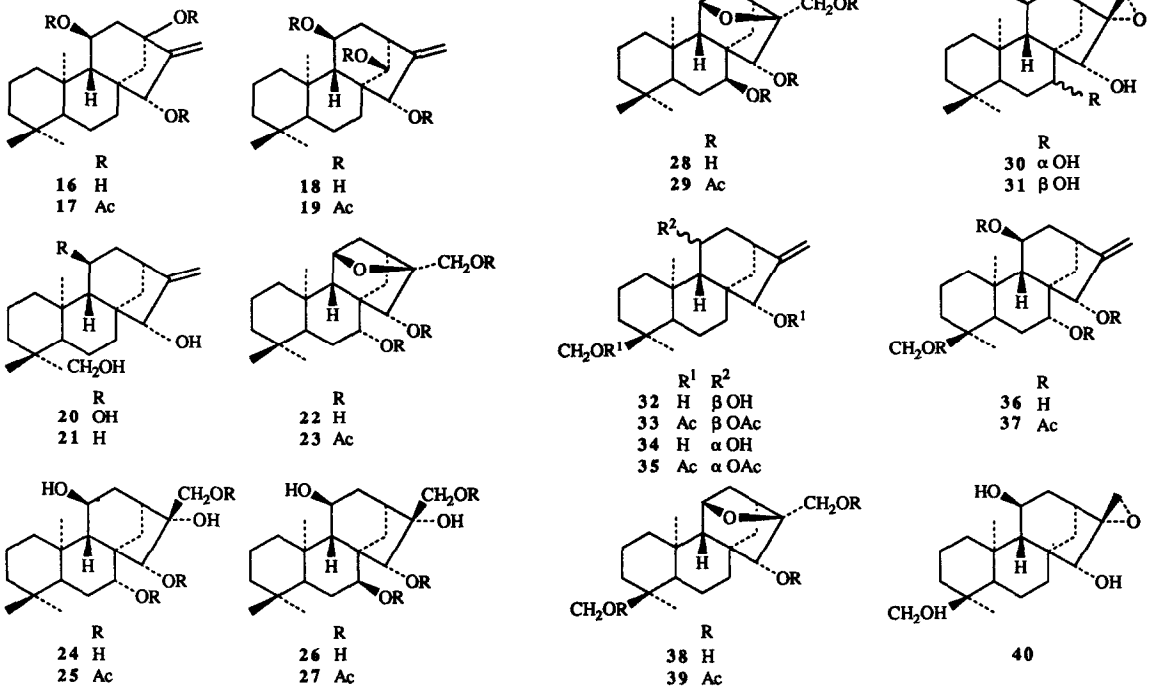
These results also indicated that the structure (**26**) assigned to a triol obtained in the microbiological transformation described above [3] must be corrected to **28**. In this case, the structure **31** must represent the alcohol produced in the biotransformation, and structure **28** the compound it is transformed to during the extraction procedure, in an analogous way to the formation of **22**.

In a previous study, we have shown that candidiol (**3**) is transformed by *G. fujikuroi* into *ent*-11α,15β,18-trihydroxy-kaur-16-ene (**32**) and an unidentified compound [1]. We have now repeated this incubation to complete this study. In this way we obtained **32** again and a mixture of compounds which was resolved by acetylation and chromatography of their acetates.

The least polar substance obtained was identified as *ent*-11β,15β,18-triacetoxy-kaur-16-ene (**35**) on the basis of the following: comparison of its ^{1}H NMR spectrum and that of candidiol diacetate (**4**) revealed the presence in **35** of a geminal proton to a new acetoxy group at δ5.10 (*m*, $W_{1/2}$ = 22 Hz), attributable to an oxygen function at C-2β, C-6β or C-11α. The last position was chosen from a consideration of the ^{13}C NMR data (Table 1). Thus, the compound obtained in this feeding experiment was *ent*-11β,15β,18-trihydroxy-kaur-16-ene (**34**).

A further compound obtained in this chromatography was *ent*-7β,11α,15β,18-tetraacetoxy-kaur-16-ene (**37**). Its ^{1}H NMR spectrum was similar to that of **35**, with the exception of a new signal assigned to the geminal proton to an acetoxy group at C-7α, which appeared as a double doublet centred at δ4.85 with coupling constants of 11 and 5 Hz. Other positions for this acetoxyl group such as C-1α and C-3α were excluded after examination of the ^{13}C NMR data (Table 1). Thus, the substance obtained in the biotransformation was **36**.

Finally, we obtained the triacetate **39** as the last product of the chromatography of the acetate mixture. Its HR mass spectrum was in accordance with the formula $C_{26}H_{38}O_7$ and the ^{1}H NMR spectrum showed two double doublets of two acetoxymethylene groups, which



were assigned to C-17 and C-18. Taking into consideration that the form of the resonance of the hydrogens at C-17 indicated that C-16 was totally substituted, and also that in this spectrum a proton appeared at δ 4.41 as a narrow triplet, as occurred in **23**, we ascribed an ether bridge between C-11β and C-16β to the structure of this product. As in the case of **23**, the alcohol **38**, corresponding to the triacetate **39**, may be an artefact formed during the acid treatment in the isolation procedure and, therefore, the biotransformed product of candidiol (**3**) should be **40**.

The biotransformations studied here confirm earlier results [1–3]. Thus, in substrates hydroxylated at C-15α there is a preference for β-hydroxylation at C-11, and, moreover, the oxidation at C-19 to the acid level is completely inhibited by the presence of the 15α-hydroxyl groups.

This microbiological transformation of 15α-hydroxy-*ent*-kaur-16-ene derivatives may be useful for the synthesis of natural 11β-hydroxy-*ent*-kaur-16-ene analogues of the type which have been obtained from liverworts of the genera *Jungermannia* [11], *Porella* [13] and *Solenostoma* [9], and from plants of the genera *Eupatorium* [12], *Helichrysum* [14], *Isodon* [15] and *Rabdosia* [16].

EXPERIMENTAL

Mps: uncorr. IR: $CHCl_3$; NMR: $CDCl_3$; MS: 70 eV (probe); CC: silica gel 0.063–0.2 mm. The substances were crystallized from petrol–EtOAc except where otherwise indicated.

Incubation experiments. Gibberella fujikuroi (ACC 917) inhibited with 5×10^{-5} MAMO 1618, was grown in shake culture at 25° for 2 days in 65–75 conical flasks (250 ml) each containing sterile medium (50 ml) [17]. The substrate (see below) in EtOH (13–15 ml) was distributed equally between the flasks and the incubation allowed to continue for a further 6 days. The broth was filtered, adjusted to pH 2 with dil. HCl, and extracted with EtOAc. The extract was sepd into acidic and neutral fractions with $NaHCO_3$. The acidic fraction was methylated with CH_2N_2, but no acidic kaurene derivatives were obtained on chromatography of the residue.

The addition of *ent*-15β-hydroxy-kaur-16-ene (**1**) (350 mg) gave in the neutral fraction: starting material (190 mg), *ent*-11α,15β-dihydroxy-kaur-16-ene (**12**) (4 mg), *ent*-7β,11α,15β-trihydroxy-kaur-16-ene (**14**) (17 mg), *ent*-11α,13,15β-trihydroxy-kaur-16-ene (**16**) (20 mg), *ent*-11α,15β,19-trihydroxy-kaur-16-ene (**20**) (4 mg), and *ent*-11α,14α,15β-trihydroxy-kaur-16-ene (**18**) (5 mg), together with a mixture of products, which was resolved by acetylation and chromatography to afford *ent*-11α,14α,15β-triacetoxy-kaur-16-ene (**19**) (2 mg) and *ent*-7β,15β,17-triacetoxy-11α,16α-epoxy-kaur-16-ene (**23**) (4 mg).

The addition of candidiol (*ent*-15β,18-dihydroxy-kaur-16-ene) (**3**) (530 mg), after chromatography of the neutral fraction and elution with mixture of petrol–EtOAc, gave: starting material (260 mg), *ent*-11α,15β,18-trihydroxy-kaur-16-ene (**32**) (70 mg), and a mixture of substances, which was resolved by acetylation and chromatography to afford *ent*-11β,15β,18-triacetoxy-kaur-16-ene (**34**) (15 mg), *ent*-7β,11α,15β,18-tetraacetoxy-kaur-16-ene (**36**) (12 mg), and *ent*-15β,17,18-triacetoxy-11α,16α-epoxy-kaurane (**38**) (4 mg).

ent-11α,15β-*Dihydroxy-kaur*-16-*ene* (**12**). $[M-H_2O]^+$ at m/z 286.2270. $C_{20}H_{30}O$ requires 286.2296; ^{1}H NMR (200 MHz): δ0.82, 0.89 and 0.92 (each 3H, *s*), 3.89 (1H, *d*, $J=5$ Hz, H-11), 4.27 (1H, *s*, H-15), 5.24 and 5.27 (each 1H, H-17); EIMS m/z (rel. int.): 286 $[M-H_2O]^+$ (10), 271 (9), 268 (1), 253 (3), 243 (2), 231 (3), 220 (2), 213 (13), 202 (4).

ent-7β,11α,15β-*Trihydroxy-kaur*-16-*ene* (**14**). ^{1}H NMR (200 MHz): δ0.82, 0.91 and 0.92 (each 3H, *s*), 2.82 (1H, *br s*, H-13), 3.92 (2H, complex signal, H-7 and H-11), 4.59 (1H, *br s*, H-15), 5.19 and 5.22 (each 1H, *s*, H-17); EIMS m/z (rel. int.): 320 $[M]^+$ (1), 302 (4), 287 (2), 284 (6), 269 (6), 266 (1), 245 (4), 241 (2), 190 (4), 187 (2). *Triacetate* **15**, $[M-HOAc]^+$ at m/z 386.2455. $C_{24}H_{34}O_4$ requires 386.2457; ^{1}H NMR (200 MHz): δ0.79, 0.88 and 1.00 (each 3H, *s*), 1.96, 1.99 and 2.06 (each 3H, *s*), 2.79 (1H, *br s*, H-13), 4.88 (1H, *dd*, $J=11$ and 5 Hz, H-7), 4.96 and 5.09 (each 1H, *s*, H-17). 5.12 (1H, H-11, signal overlapped with one H-17), 5.89 (1H, *s*, H-15); ^{1}H NMR (200 MHz, C_6D_6): δ0.70, 0.72 and 0.81 (each 3H, *s*), 1.82, 1.84 and 1.96 (each 3H, *s*), 5.09 and 5.26 (each 1H, *s*, H-17), 5.10 (1H, H-11, overlapped with one H-17), 5.17 (1H, *dd*, $J=11$ and 5 Hz, H-7), 5.26 (1H, *s*, H-15); EIMS m/z (rel. int.): 386 $[M-HOAc]^+$ (2), 344 (5), 326 (10), 311 (3), 302 (3), 284 (24), 266 (9), 251 (8), 240 (3), 227 (3).

ent-11α,13,15β-*Trihydroxy-kaur*-16-*ene* (**16**). Mp 198–200°, $[M]^+$ at m/z 320.2340. $C_{20}H_{32}O_3$ requires 320.2355; ^{1}H NMR (200 MHz): δ0.82, 0.89 and 0.93 (each 3H, *s*), 4.10 (1H, *d*, $J=5.5$ Hz, H-11), 4.30 (1H, *s*, H-15), 5.35 (2H, *s*, H-17); EIMS m/z (rel. int.): 320 $[M]^+$ (1), 302 (5), 287 (2), 284 (8), 269 (6), 241 (2), 205 (2), 199 (3). *Triacetate* **17**, $[M-HOAc]^+$ at m/z 386.2460. $C_{24}H_{34}O_4$ requires 386.257; ^{1}H NMR (200 MHz): δ0.81, 0.85 and 1.03 (each 3H, *s*), 1.94, 2.04 and 2.09 (each 3H, *s*), 2.81 (2H, *m*, H-12), 5.20 (1H, *d*, $J=5.5$ Hz, H-11), 5.22 and 5.26 (each 1H, *s*, H-17); 5.57 (1H, *s*, H-15); EIMS m/z (rel. int.): 386 $[M-HOAc]^+$ (5), 344 (8), 326 (14), 311 (15), 284 (27), 269 (7), 266 (6), 251 (5), 229 (9), 202 (8).

ent-11α,14α,15β-*Trihydroxy-kaur*-16-*ene* (**18**). Mp 228–230°; $[M]^+$ at m/z 320.2328. $C_{20}H_{32}O_3$ requires 320.2353; ^{1}H NMR (200 MHz): δ0.83, 0.89 and 1.10 (each 3H, *s*), 3.72 (1H, *s*, H-15), 3.77 (1H, *d*, $J=3.5$ Hz, H-11), 4.05 (1H, *s*, H-14), 5.28 and 5.37 (each 1H, *s*, H-17); EIMS m/z (rel. int.): 320 $[M]^+$ (1), 302 (11), 287 (6), 284 (8), 269 (12), 266 (1), 255 (3), 251 (2), 245 (3), 229 (3). *Triacetate* **19**, $[M-C_2H_2O]^+$ at m/z 404.2563. $C_{24}H_{36}O_5$ requires 404.2562; ^{1}H NMR (200 MHz): δ0.83, 0.86 and 1.13 (each 3H, *s*), 1.97 and 2.08 (each 3H, *s*), 2.94 (1H, *br s*, H-13), 4.61 (1H, *d*, $J=3.5$ Hz, H-11), 4.94 (1H, *s*, H-15), 5.24 and 5.30 (each 1H, *s*, H-17), 5.48 (1H, *s*, H-14); EIMS m/z (rel. int.): 404 $[M-C_2H_2O]^+$ (1), 386 (2), 371 (3), 344 (6), 326 (20), 311 (21), 302 (2), 284 (48), 269 (11), 266 (15), 255 (3), 251 (16), 229 (8), 213 (5), 202 (8).

ent-7β,15β,17-*Triacetoxy*-11α,16α-*epoxy-kaurane* (**23**). $[M-C_2H_2O]^+$ at m/z 420.2518. $C_{24}H_{36}O_6$ requires 420.2511; IR ν_{max} cm^{-1}: 2930, 1730, 1375, 981; ^{1}H NMR (200 MHz): δ0.82, 0.87 and 1.15 (each 3H, *s*), 1.95, 2.02 and 2.05 (each 3H, *s*), 3.97 and 4.49 (each 1H, *d*, $J=12$ Hz, H-17), 4.43 (1H, *t*, H-11), 5.02 (1H, *dd*, $J=11$ and 5 Hz, H-7), 5.15 (1H, *s*, H-15); EIMS m/z (rel. int.): 420 $[M-C_2H_2O]^+$ (1), 402 (3), 360 (4), 342 (26), 300 (12), 282 (19), 267 (7), 249 (11), 239 (7), 228 (14), 207 (23).

ent-11β,15β,18-*Triacetoxy-kaur*-16-*ene* (**35**). $[M-HOAc]^+$ at m/z 386.2416. $C_{24}H_{34}O_4$ requires 386.2457; ^{1}H NMR (200 MHz): δ0.88 and 1.08 (each 3H, *s*), 1.99, 2.00 and 2.06 (each 3H, *s*), 2.71 (1, *br s*, H-13), 3.63 and 3.94 (each 1H, *d*, $J=11$ Hz, H-18), 5.10 (1H, *m*, $W_{1/2}=22$ Hz, H-11), 5.09 and 5.21 (each 1H, *s*, H-17), 5.21 (1H, *br s*, H-15); ^{1}H NMR (200 MHz, C_6D_6): δ0.85 and 0.88 (each 3H, *s*), 1.71, 1.83 and 1.97 (each 3H, *s*), 2.57 (1H, *br s*, H-13), 3.90 and 4.11 (each 1H, *d*, $J=11$ Hz, H-18), 5.11 and 5.56 (each 1H, *s*, H-17), 5.38 (1H, *m*, $W_{1/2}=22$ Hz, H-11), 5.53 (1H, *s*, H-15); EIMS m/z (rel. int.): 386 $[M-HOAc]^+$ (1), 344 (3), 326 (23), 311 (8), 283 (10), 270 (19), 266 (20), 253 (23), 251 (40), 238 (10), 225 (12), 223 (14), 210 (22), 197 (18).

ent-7β,11α,15β,18-*Tetraacetoxy-kaur*-16-*ene* (**37**). $[M-HOAc]^+$ at m/z 444.2528. $C_{26}H_{36}O_6$ requires 444.2510; ^{1}H NMR (200 MHz): δ0.79 and 1.03 (each 3H, *s*), 1.98, 1.99, 2.04 and 2.11 (each 3H, *s*), 2.80 (1H, *br s*, H-13), 3.60 and 3.81 (each 1H, *d*, $J=11$ Hz, H-18), 4.85 (1H, *dd*, $J=11$ and 5 Hz, H-7), 4.96 and 5.10 (each 1H, *s*, H-17), 5.11 (1H, *br s*, H-11), 5.92 (1H, *s*,

H-15); ^{1}H NMR (200 MHz, C_6D_6): δ 0.58 and 0.67 (each 3H, *s*), 1.76, 1.78, 1.88, 1.90 (each 3H, *s*), 2.53 (1H, *br s*, H-13), 3.58 and 3.91 (each 1H, *d*, $J = 11$ Hz, H-18), 5.07 and 5.21 (each 1H, *s*, H-17), 5.15 (2H, *br s*, H-7 and H-11), 6.26 (1H, *br s*, H-15); EIMS *m/z* (rel. int.): 444 $[M-HOAc]^+$ (1), 402 (15), 384 (12), 342 (38), 324 (12), 282 (39), 269 (16), 267 (22), 264 (12), 253 (12), 251 (20), 239 (8), 225 (9), 211 (10), 198 (12).

ent-15β,17,18-*Triacetoxy*-11α,16α-*epoxy-kaurano* (**39**). $[M]^+$ at *m/z* 462.2615. $C_{26}H_{38}O_7$ requires 462.2603; IR ν_{max} cm^{-1}: 2930, 1725, 1370; ^{1}H NMR (200 MHz): δ0.82 and 1.13 (each 3H, *s*), 2.00, 2.03 and 2.04 (each 3H, *s*), 3.57 and 3.80 (each 1H, *d*, $J = 11$ Hz, H-18), 3.96 and 4.51 (each 1H, *d*, $J = 12$ Hz, H-17), 4.41 (1H, *t*, H-11), 4.98 (1H, *s*, H-15); EIMS *m/z* (rel. int.): 462 $[M]^+$ (0.2), 444 (0.7), 402 (3), 384 (2), 360 (1), 342 (10), 329 (7), 282 (1), 269 (9), 264 (4), 255 (3), 213 (3), 211 (4), 199 (4).

Partial hydrolysis of candidiol diacetate (**4**). A soln of **4** (1 g) in MeOH (3 ml) was treated with 2% methanolic KOH (15 ml) and the mixture left at room temp. for 45 min. Usual work-up and chromatography with petrol–EtOAc (4:1) as eluent afforded, besides starting material (290 mg), a mixture of the 18-monoacetate **6** and 15α-monoacetate **5** (490 mg) and candidiol (**3**) (115 mg).

The mixture of monoacetates was chromatographed on a dry column of silica gel impregnated with 15% $AgNO_3$. Elution with petrol–EtOAc (4:1) gave ent-15β-acetoxy-18-hydroxy-kaur-16-ene (**5**) (320 mg). Mp 142–144°; $[M]^+$ at *m/z* 346.2505. $C_{22}H_{34}O_3$ requires 346.2506; ^{1}H NMR (200 MHz): δ0.74 and 1.05 (each 3H, *s*), 2.03 (3H, *s*), 3.09 and 3.38 (each 1H, *d*, $J = 11$ Hz, H-18), 5.07 (1H, *s*, H-15), 5.09 and 5.24 (each 1H, *s*, H-17); EIMS *m/z* (rel. int.): 346 $[M]^+$ (1), 331 (3), 304 (5), 286 (28), 271 (37), 259 (5), 256 (31), 255 (91), 241 (15), 227 (7), 213 (9), 211 (7), 199 (9), 187 (12), 185 (18), 175 (11), 173 (33), 161 (18), 160 (12), 159 (31), 157 (17), 149 (16), 147 (35), 145 (37), 143 (22), 135 (31), 123 (50). Further elution afforded ent-18-acetoxy-15β-hydroxy-kaur-16-ene (**6**) (170 mg); ^{1}H NMR (200 MHz): δ0.85 and 1.05 (each 3H, *s*), 2.05 (3H, *s*), 3.62 and 3.87 (each 1H, *d*, $J = 11$ Hz, H-18), 3.81 (1H, *s*, H-15), 5.06 and 5.20 (each 1H, *s*, H-17).

ent-15β-*Acetoxy*-18-*chloro-kaur*-16-*ene* (**7**). To the monoacetate **5** (320 mg) in dry pyridine (6 ml) and CCl_4 (30 ml), triphenylphosphine (560 mg) was added and the mixture refluxed for 4 hr. Extraction with EtOAc in the usual way and chromatography of the residue, eluting with petrol–EtOAc (19:1) afforded **7**; ^{1}H NMR (80 MHz): δ 0.87 and 1.05 (each 3H, *s*), 2.03 (3H, *s*), 3.18 and 3.43 (each 1H, *d*, $J = 11$ Hz, H-18), 5.09 (1H, *br s*, H-15), 5.09 and 5.25 (each 1H, *s*, H-17).

Hydrolysis of compound **7**. The monoacetate **7** (330 mg) in MeOH was treated with methanolic KOH (5%) (10 ml) at room temp. and left overnight. Usual work-up gave ent-15β-hydroxy-18-chloro-kaur-16-ene (**8**) (295 mg); mp 91–93°; $[M]^+$ at *m/z* 322.2069. $C_{20}H_{31}OCl$, requires 322.2062; ^{1}H NMR (80 MHz): δ0.88 and 1.05 (each 3H, *s*), 3.19 and 3.47 (each 1H, *d*, $J = 11$ Hz, H-18), 3.81 (1H, *br s*, H-15), 5.06 and 5.19 (each 1H, *s*, H-17); EIMS *m/z* (rel. int.): 322 $[M]^+$ (9), 307 (18), 304 (11), 291 (13), 289 (34), 273 (12), 266 (31), 264 (100), 255 (19), 249 (56), 247 (32), 240 (4), 239 (18), 225 (6), 185 (13), 183 (15).

Reduction of compound **8**. Compound **8** (295 mg) in dry C_6H_6 (10 ml) was added dropwise to a refluxing soln of tri-*n*-butyl tin hydride (0.9 ml) and azabisisobutyronitrile (trace) in dry C_6H_6 (7 ml). The mixture was allowed to reflux for a further 4 hr, when the solvent was evapd and the residue dissolved in Et_2O, an aq. soln of KF was added and the ppt. sepd by filtration. The Et_2O fraction was dried (Na_2SO_4) and the solvent evapd. Chromatography of the residue, eluting with petrol–EtOAc (19:1) afforded ent-15β-hydroxy-kaur-16-ene (**1**) (190 mg). Mp 109–110° (MeOH), $[M]^+$ at *m/z* 288.2499. $C_{20}H_{32}O$ requires 288.2452; ^{1}H NMR (200 MHz): δ 0.81, 0.86 and 1.02 (each 3H, *s*), 2.72 (1H, *br s*, H-13), 3.80 (1H, *s*, H-15), 5.06 and 5.19 (each 1H, *s*, H-17); EIMS *m/z* (rel. int.): 288 $[M]^+$ (11), 273 (33), 270 (10), 255 (34), 230 (37), 215 (22), 213 (13), 205 (9), 203 (7), 189 (9), 177 (9), 173 (11), 163 (12).

ent-19-*Chloro-kaur*-16-*ene* (**10**). Treatment of *ent*-19-hydroxy-kaur-16-ene (**9**) (350 mg) in dry pyridine (7 ml) and CCl_4 (30 ml) with triphenylphosphine (550 mg) at reflux for 6 hr gave, in the same way as above for **5**, compound **10** (290 mg); mp 75–77°; $[M]^+$ at *m/z* 308.2127. $C_{20}H_{31}Cl^{37}$ requires 308.2082; $[M]^+$ at *m/z* 306.2112. $C_{20}H_{31}Cl^{35}$ requires 306.2113; ^{1}H NMR (80 MHz): δ 1.03 (6H, *s*), 3.38 and 3.87 (each 1H, *d*, $J = 11$ Hz, H-19), 4.79 (2H, *br s*, H-17); EIMS *m/z* (rel. int.): 308 $[M]^+$ (6), 306 $[M]^+$ (18), 293 (14), 291 (43), 265 (10), 263 (26), 257 (50), 249 (9), 247 (21), 227 (8), 203 (4), 201 (6), 189 (3), 187 (10), 173 (9), 171 (17), 161 (15), 159 (23).

Reduction of compound **10**. Compound **10** (290 mg) was treated with tri-*n*-butyl tin hydride in the same way as above for **8** to give ent-*kaur*-16-*ene* (**11**) (188 mg); ^{1}H NMR (200 MHz): δ 0.81, 0.85 and 1.02 (each 3H, *s*), 2.64 (1H, *br s*, H-13), 4.73 and 4.79 (each 1H, *s*, H-17); EIMS *m/z* (rel. int.): 272 $[M]^+$ (21), 257 (70), 229 (34), 213 (19), 203 (7), 201 (10), 187 (19).

Allylic oxidation of compound **11**. Compound **11** (185 mg) in dioxane (5 ml) was treated with SeO_2 (45 mg) and H_2O (3 ml) for 7 hr at room temp. The soln was poured into H_2O and extracted with EtOAc in the usual way. Chromatography of the residue gave *ent*-15β-hydroxykaur-16-ene (**1**) (102 mg).

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REFERENCES

1. Fraga, B. M., González, P., Hernández, M. G., Tellado, F.G. and Perales, A. (1986) *Phytochemistry* **25**, 1235.
2. Fraga, B. M., Hernández, M. G., Díaz, C. E., González, P. and Guillermo, R. (1988) *Phytochemistry* **27**, 3131.
3. Fraga, B. M., Hernández, M. G. and González, P. (1991) *Phytochemistry* **30**, 2567.
4. MacMillan, J. (1980) *Hormonal Regulation of Development, Molecular Aspects of Plant Hormones*. Springer, Berlin.
5. Rodríguez, B., Valverde, S. and Rocha, J. M. (1970) *An. Quim.* **66**, 503.
6. González, A. G., Fraga, B. M., Hernández, M. G., Luis, J. G. and Larruga, F. (1979) *Biochem. Syst. Ecol.* **7**, 115.
7. Dennis, D. T., Upper, C. D. and West, C. A. (1965) *Plant Physiol.* **40**, 948.
8. Cross, B. E. and Myers, P. L. (1969) *Phytochemistry* **8**, 79.
9. Connolly, J. D. and Thornton, I. M. S. (1973) *J. Chem. Soc., Perkin Trans. I* 736.
10. Tanaka, N., Nakatani, K., Murakami, T., Saiki, Y. and Chen, C. M. (1978) *Chem. Pharm. Bull.* **26**, 3260.
11. Benes, I., Benesová, V. and Herout, V. (1977) *Collect. Czech. Chem. Commun.* **42**, 1229.
12. Herz, W. and Sharma, R. P. (1976) *J. Org. Chem.* **41**, 1021.
13. Matsuo, A., Uto, S., Nakayama, M. and Hayashi, S. (1977) *Chem. Letters* 327.
14. Bohlmann, F., Zdero, C., Hoffmann, E., Mahanta, P. K. and Dorner, W. (1978) *Phytochemistry* **17**, 1917.
15. Wang, Z. M., Cheng, P. Y., Zheng, Q. T., Wu, C. Y., Xu, M. J., Gue, Y. W., Mizuno, M., Iinuma, M. and Tanaka, T. (1991) *Phytochemistry* **30**, 3699.
16. Chen, Y. Q., Shi, X. L. and Zheng, P. J. (1991) *Phytochemistry* **30**, 917.
17. Hanson, J. R., Hawker, J. and White, A. F. (1972) *J. Chem. Soc., Perkin Trans. I* 1982.